at an equivalent position to cathepsin E. Asymmetrical, similar, pH-activity curves were obtained for both these proteinases, with the maxima at pH 3.4 to 3.5. After agar-gel electrophoresis, both proteinases were located towards the anode with proteinase 2b slightly ahead of proteinase 2a. Proteinase 1 was resolved further into two major (1a and 1b) and two minor peaks when chromatographed on CM-cellulose.

The sites of action of proteinases 1a and 1b on the B-chain of oxidized insulin were investigated at pH 3.5. Both cathepsins cleaved to completion bonds Tyr₍₁₆₎-Leu₍₁₇₎ and Phe₍₂₄₎-Phe₍₂₅₎ and both exhibited moderate action at bonds Leu₍₁₅₎-Tyr₍₁₆₎ and Phe₍₂₅₎-Tyr₍₂₆₎. Bonds Phe₍₁₎-Val₍₂₎, Glu₍₁₃₎-Ala₍₁₄₎ and Ala₍₁₄₎-Leu₍₁₅₎ were partially cleaved by proteinase 1a and only weakly attacked by proteinase 1b, whereas the bond at Gly₍₂₃₎-Phe₍₂₄₎ was more susceptible to hydrolysis by proteinase 1b.

Proteinases 1a and 1b have therefore similar specificities, and in their action they resemble two cathepsins D that have been previously investigated from bovine lung (Dannenberg & Smith, 1955) and swine thyroid (Kress, Peanasky & Klitgaard, 1966). Proteinases 2a and 2b have not been purified sufficiently for an investigation of their respective specificities.

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Regulation of Penicillinase Synthesis in *Bacillus licheniformis*: Binding of Inducer to Cells, and the Half-Life of the Messenger Ribonucleic Acid

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When cells of *Bacillus licheniformis* 749 are induced with penicillin, there is a slow increase in the differential rate of synthesis of penicillinase, the maximum rate being reached 60 min. or more after induction. This phenomenon, and the very slow deadaptation rate on removal of inducer, have encouraged speculation that the regulation mechanism may differ from other inducible enzymes.

Experiments interpreted as evidence for a long-lived messenger RNA led Harris & Sabath (1964) to suggest that the scheme proposed by Jacob & Monod (1961) is not applicable to the regulation of penicillinase in *Bacillus cereus*. An alternative model has been proposed (Csanyi, Jacobi & Straub, 1967) involving stabilization of the messenger after addition of inducer. It has been suggested that such a model can apply also to *B. licheniformis* (see Richmond, 1969) and that the slow deadaptation of penicillinase synthesis is a consequence of the long life of stabilized message. The binding of the inducer, [¹⁴C]penicillin, to *B. licheniformis* cells has been measured and the results showed a specific, irreversible, binding as in *B. cereus* (Pollock & Perret, 1951; Duerksen, 1963) and *Staphylococcus aureus* (Rogers, 1967), although 20–30-fold higher (approx. 5000 molecules/cell). Deadaptation by resuspending washed cells in a fresh medium does not remove the bound inducer, and thus the slow deadaptation can be explained without involving long-lived messenger. The existence of long-lived messenger has also been challenged. The enzyme-forming capacity of *B. licheniformis* cells was measured after stopping transcription with actinomycin D or rifampicin (1–5 µg./ml.). The half-life of the penicillinase messenger was estimated to be 4.7 min. for induced or constitutive cells. Although this value is twice the average half-life for total messenger RNA in this organism, there was no indication of an unusually long-lived messenger.

It is suggested that regulation of penicillinase production in *B. licheniformis* does not differ fundamentally from the β-galactosidase system of *Escherichia coli*. However, since the binding of inducer may be at the cell surface, it may require a secondary internal inducer to remove repressor from the DNA. Alternatively, if penicillin is the primary inducer, there may be a physical separation between inducer and repressor, which could explain the induction kinetics. A model suggesting this type of regulation, based on experiments with *B. cereus*, has recently been proposed (Imsande, 1969) and would seem to apply to *B. licheniformis*.

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